5-2009

Understanding the effects of hydrophobicity for the detection of methylated DNA by microchannel electrophoresis

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Understanding the Effects of Hydrophobicity for the Detection of Methylated DNA by Microchannel Electrophoresis

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May 1, 2009

DNA methylation is an epigenetic mechanism used for long – term silencing of gene expression. This silencing of gene expression can be involved in the down regulation of genes involved in apoptosis, which is a process of regulated cell death, and this inability to break down cells can ultimately lead to tumor formation in cancer. Methylation of DNA follows a certain pattern through developmental stages of an individual, and this pattern is normally maintained throughout that individual's life. However, in the DNA of older individuals, this methylation is commonly seen to be altered. This altered pattern of methylation can be important in initiating tumorigenesis and sustaining the malignant state of cancer cells[9]. The potential reversibility of DNA methylation patterns suggests a very viable target for the treatment of cancer.

This study examines the effectiveness of an alternate method of detection of these altered DNA methylation patterns through microchannel electrophoresis. This method detects DNA methylation by using the increased hydrophobicity of methylated DNA to allow it to be separated from non – methylated DNA in capillary electrophoresis (CE). This separation is accomplished by using a hydrophobic copolymer as a separation medium in CE. The hydrophobic interactions that result between the copolymer and methylated DNA can lead to a slower elution time of the DNA through the capillaries during CE. This slower elution time of methylated DNA can allow it to be distinguished from non-methylated DNA; therefore, resulting in the effective detection of this alteration. In this project, copolymers of varying degrees of hydrophobicity are tested to determine the potential of this method to provide effective detection of DNA methylation.

Introduction

DNA Methylation:

DNA methylation is one of the most studied epigenetic phenomena due to the key role it has been shown to have in carcinogenesis and tumor progression.[9] 'Epigenetic' is a term used to describe mitotically and meiotically heritable states of gene expression that do not result in changes in the DNA sequence, and this epigenetic event can result in the long – term silencing of gene expression.[9] The human genome possesses a normal methylation pattern; however, a deviation of the extent of methylation from this normal pattern can lead to altered gene expression. Detection of this epigenetic alteration is of particular interest due to the potential reversibility of DNA methylation patterns, which suggests a viable target for treatment of certain types of cancer.[9] There are currently several different methods that exist for the detection of DNA methylation, but these methods contain many drawbacks. Developing a more efficient and effective method for the detection of altered DNA methylation patterns could potentially lead to more successful treatments of cancer.

The silencing of gene expression through DNA methylation can be involved in the down regulation of genes involved in apoptosis, which is a process of regulated cell death. This inability to break down cells ultimately leads to tumor formation in cancer.[10] Methylation of DNA follows a certain pattern through the developmental stages of an individual, and this pattern is normally maintained throughout that individual's life. However, in the DNA of older individuals, this methylation is commonly seen to be altered. This altered pattern of methylation can be important in initiating tumorigenesis and sustaining the malignant state of cancer cells.[9]

In humans, DNA methylation is the only known natural modification of DNA, and it only affects the cytosine (C) base of DNA when it is followed by a guanine (G). This modification

usually only occurs in areas of human DNA in which CpG density is relatively low; ~3-4% of all cytosines are methylated in normal human DNA.[4] Approximately 50% of genes are associated with a high frequency of CG bases in their promoter regions, and these areas, known as "CpG" islands, are usually low in methylation and capable of transcriptional activation.[9] These islands are ~200-1000 base pairs in length and often coincide with the 5` ends of genes,[10] and the genes in these areas that control cell replication can be silenced by DNA methylation, which results in cancerous tumor formation by inhibiting apoptosis.[9]

There are currently several methods for detection of DNA methylation including bisulfite conversion of DNA followed by sequencing, methylation-sensitive restriction enzyme digestion followed by a Southern blot, and restriction landmark genomic scanning (RLGS).[10] Many of these methods have proven effective, but there are still many drawbacks, such as difficulty in obtaining complete conversion in the bisulfite step and the use of harsh chemicals, and these methods are discussed in detail in the "Previous Studies" section of this paper.[10] The purpose of this research is to find more rapid and effective methods of detection of DNA methylation. This project investigates the potential for detection of DNA methylation based on the increased hydrophobicity of hypermethylated DNA by using varying degrees of hydrophobic polymer as a separation medium in capillary electrophoresis.

Electrophoresis:

Electrophoresis is the use of an electric field to separate charged molecules based on a difference in electrophoretic mobility, which can result from differences in charge and/or shape or size. Capillary electrophoresis (CE) separates charged or uncharged molecules in a thin, buffer-filled capillary by the application of a very high voltage (1-30 kV).[8] Because DNA

possesses an overall negative charge, the potential difference through the capillaries causes the DNA molecules to migrate from the cathode to the anode. In the analysis of DNA by capillary electrophoresis, the migration time and relative sample quantity are the basic parameters obtained from a CE electropherogram. The migration abilities of DNA through the capillaries can be manipulated using various separation mediums, which are selected based on the DNA characteristics being measured such as fragment size or hydrophobicity.

The beneficial characteristics of CE result from an extremely sensitive technique, fast separations (< 5 minutes), and consistent reproducibility studies showing the coefficient of variation to be <2%.[8] The inner wall of a fused silica capillary is negatively charged when in contact with basic buffers. The wall attracts a thin layer of cations of thickness $\lambda_D \cong$ 1-10 nm, which is termed the Debye length.[7] In the presence of an electric field, the diffuse part of this layer moves and drags the liquid toward the cathode, which produces electroosmotic flow (EOF). In this research, it is desirable to suppress the EOF because the "resulting axial flow gradient may affect the resolution of analytes."[7] The effective mobility of the analyte is represented by the equation:[7]

$$\mu = \mu_{electrophoretic} + \mu_{EOF} \quad (1)$$

where $\mu =$ observed mobility, $\mu_{electrophoretic}$= electrophoretic mobility contribution, and μ_{EOF}= the EOF contribution. Capillary wall coating agents can be used to eliminate the EOF mobility contribution. Poly-N-hydroxyethylacrylamide (PHEA) was chosen for this research because it "exhibits good capillary-coating ability, *via* adsorption from aqueous solution, efficiently suppressing electroosmotic flow (EOF)."[5]

In order to detect DNA methylation using capillary electrophoresis, the increased hydrophobicity of methylated DNA can be targeted. This research utilizes a separation medium

of polymers made from monomers of increasing hydrophobicity. DNA possesses an overall negative charge, and an increase in the extent of methylation of the DNA leads to an increase its degree of hydrophobicity. The presence of the hydrophobic groups in the polymer separation matrix should decrease the mobility of the methylated DNA because of its hydrophobic interactions with the separation polymer. This separation should allow the extent of the DNA methylation within a given sequence to be observed.

Significance:

As stated before, the epigenetic mutation of DNA methylation has a high potential of reversibility. This reversibility suggests a feasible target for cancer treatment, and this treatment can be accomplished through the use of demethylating agents. Treatment of cancer cells with demethylating agents can reactivate a group of genes such as p16, mutL homologue – 1 (MLH1) and retinoblastoma (RB) that are often crucial in controlling cell proliferation, differentiation, apoptosis and other key homeostatic mechanisms.[9] The re-activation of genes in cancer cells that control apoptosis allows the cell to regain its normal ability to break down, therefore suppressing tumor formation in cancer. Developing more efficient and effective detection methods of altered DNA methylation patterns in humans has the potential to lead to more effective methods of cancer treatment and could also aid in the ultimate goal of cancer prevention.

Previous Studies:

There are currently several methods that exist for detection of DNA methylation. The explosion of studies involving changes in DNA methylation has only happened within the last

ten years due to increasing awareness of the importance of epigenetic silencing by DNA methylation in cancer.[3] Selected methods will be discussed in further detail below.

One commonly used method that is considered to be the gold standard for detection of DNA methylation is bisulfite modification followed by sequencing. When DNA is treated with sodium bisulfite under denaturing conditions, all cytosines are converted into uracil residues, which are recognized as thymines in subsequent PCR amplification.[6] All methylated cytosine residues are protected from this reaction and remain unchanged, thus, the method allows direct and positive determination of methylation sites in the genomic DNA, as only methylated cytosines are detected as cytosines.[6] The products of PCR-amplified bisulphite-treated DNA can be used directly for sequencing in order to determine an average degree of methylation, or they can be cloned and sequenced individually in order to determine the exact methylation pattern.[6] This method has a high sensitivity, ability to detect single-molecule methylation patterns, and the possibility of addressing nonsymmetrical methylation making bisulphite-based genomic sequencing very effective for DNA methylation detection.[6] There are also several drawbacks associated with this method, which include difficulty in obtaining complete conversion in the bisulfite step, PCR biasing, and the presence of artifacts in lower quality DNA. PCR biasing is a result of either the low or highly methylated template DNA being predominantly amplified due to the choice of primers.[6]

Another method, methylation-sensitive restriction fingerprinting (MSRF), involves the use of methylation-sensitive restriction enzymes to allow the methylation status of CpG sites throughout the entire DNA genome to be analyzed. MSRF is a PCR-based technique that, by virtue of the properties of methylation-sensitive restriction enzymes, is biased towards the study of CpG sites.[6] This method has been used to study the methylation status of CpG sites in

samples derived from patients with chronic myeloid leukemia, leukemia cell lines, and in the study of breast carcinomas.[6] The drawback of this method is a result of using the Southern blotting technique in order to confirm results. Southern blots require a significant amount of DNA, cannot be used on fixed samples, and require significant time and effort for detection.

In addition to these two methods, restriction landmark genomic scanning (RLGS) and methylated CpG island amplification (MCA) are commonly used methods for DNA methylation detection. RLGS is a method that provides both a quantitative genetic and epigenetic (cytosine methylation) assessment of thousands of CpG islands in a single gel without prior knowledge of gene sequence.[6] This approach has been used to identify novel tumor-specific targets of DNA amplification, aberrant CpG island methylation, and repetitive sequences that are demethylated in human cancer and in experimentally induced rodent tumors.[6] The drawback of this method is difficulty in distinguishing whether a loss of a fragment from an RLGS profile is a result of deletion or methylation.[6] In order to confirm this result, Southern blotting is required. MCA involves the amplification of closely spaced methylated SmaI restriction sites to enrich for methylated CpG islands. The advantages of this method are that a large number of samples can be analyzed rapidly at multiple loci, many steps of MCA can be readily automated, it allows for an unbiased representation of CpG islands without requiring prior knowledge of their DNA sequence, and novel differentially methylated CpG islands can be amplified and cloned relatively simply, without the need for acrylamide gels or two-dimensional gel electrophoresis.[6] However, the disadvantages of MCA are that it is sensitive to partial digestion with restriction enzymes, it examines only a limited number of CpG sites within CpG islands, and false-positives can result from incomplete digestion using the methylation-sensitive restriction-enzyme SmaI.[6]

So far, no methods have been developed that utilize hydrophobic polymer in capillary electrophoresis to target more hydrophobic methylated DNA in order to distinguish it from non-methylated DNA. This detection method has the potential improve upon the many deficiencies of currently used methods by decreasing time needed for detection, limiting the amount of DNA required for each test, eliminating the use of harsh chemicals, and eliminating the adverse effects of PCR biasing.

Materials and Methods:

In order to study the detection of the methylation status of DNA, a sequence length of 215 base pairs was chosen for this study. This length was chosen because synthetic DNA was used, and it corresponds to the average sequence length that the primers used in this study code for in genomic Jurkat DNA. The sequences of DNA that were analyzed differed only in their degree of methylation. These sequences were obtained from Bio Basic Inc.

PCR:

The synthetic DNA sequences were amplified via polymerase chain reaction (PCR) in order to obtain an adequate amount of DNA to be used in the methylation reaction. The 20 µl PCR reaction mixture contained 4 µl 5X PCR Buffer, 1.2 µl 25 mM $MgCl_2$, 0.4 µl 10 mM deoxyunspecified nucleoside 5`-triphosphates (dNTPs), 0.5 µl 20 µM of each primer, 0.2 µl Taq polymerase, 8.2 µl of $dH_2O$, and 5 µl of DNA. The forward primer sequence used was 5`- GAC CCA AGG AGT CTA AAG GAA ACT CTA ACT – 3`, and the reverse primer sequence used was 5` - CTG ATC TTC AGA TGA TCA GAA CAA TGT GCT – 3`.[1] The temperature profiles for the amplification were 4 min at 94^{o}C, 25 cycles of 1 min at 94^{o}C, 1 min at 58^{o}C, and 1 min at

72°C, followed by a final extension of 7 min at 72°C. The PCR products were then loaded onto a 2.5% agarose gel in order to confirm that amplification did indeed occur. An electric field of 0.92 to 1.04 V was applied to the slab gel in a buffer solution of 0.5X TBE, and the development of DNA bands was detected using a short wave UV light. The PCR products were then purified in order to remove primers, nucleotides, polymerases, and salts. This was done using the MinElute PCR Purification Kit Protocol obtained from QIAGEN, and this kit is capable of purifying DNA fragments ranging from 70 bp to 4 kb using MinElute spin columns in a microcentrifuge. Once the DNA was purified, the concentration was determined using a NanoDrop instrument.

Methylation:

After a minimum of 1000 ng of amplified DNA was obtained, it was methylated using the BamHI methyltransferase enzyme. This enzyme methylates DNA at a specific site that corresponds to the recognition site of the BamHI endonuclease enzyme. The restriction site along with the corresponding locations of methylation and cleavage by BamHI methyltransferase and BamHI endonuclease respectively can be seen in Figure 1.

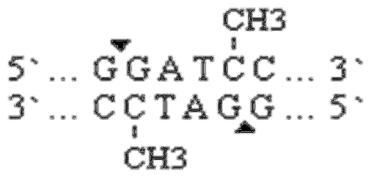

Figure 1: Recognition site of BamHI. The arrows correspond to the sites of cleavage by BamHI, and the sites of methylation of the methytransferase enzyme are shown by the methyl groups.

When DNA containing this recognition site is subjected to the BamHI methyltransferase enzyme, the cytosine residues observed in Figure 1 are methylated. As a result, the BamHI endonuclease enzyme is no longer able to cleave at this site; therefore, the methylated DNA strand remains intact if it is subjected to BamHI after methylation. This method was used to confirm that the methylation reaction was successful, and the protocols for the BamHI methyltransferase and BamHI enzyme reactions were obtained from New England BioLabs.

The sequences used in this project can be observed in Figure 2. Each of these sequences is 215 base pairs in length and contains a different number of BamHI recognition sites resulting in varying degrees of methylation between the sequences. All of these sequences were amplified via PCR; however, CE results were only obtained for the sequence containing one methylation site.

Unmethylated:
5` - gacccaagga gtctaaagga aactctaact acaacaccc aaatgccaca aaaccttagt tattaatac aaactatcat ccctgcctat ctgtcaccggatccatctca tcttaaaaaa cttgtgaaaa tacgtaatcc tcaggagact tcaattaggt ataaatacca gcagccagag gaggtgcagcacattgttct gatcatctga agatcag – 3`

1 Methylation Site:
5` - gacccaagga gtctaaagga aactctaact acaacaccc aaatgccaca aaaccttagt tattaatac aaactatcat ccctgcctat ctgtcaccggatccatctca tcttaaaaaa cttgtgaaaa tacgtaatcc tcaggagact tcaattaggt ataaatacca gcagccagag gaggtgcagcacattgttct gatcatctga agatcag – 3`

2 Methylation Sites:
5` - gacccaagga gtctaaagga aactctaact acaacaccc aaatgccaca aaaggatccttagt tattaatac aaactatcat ccctgcctat ctgtcaccatctca tcttaaaaaa cttgtgaaaa tacgtaat ggatcctcaagact tcaattaggt ataaatacca gcagccagag gaggtgcagcacattgttct gatcatctga agatcag – 3`

3 Methylation Sites:
5` - gacccaagga gtctaaagga aactctaact acaacacggatcc aaatgaca aaaccttagt tattaatac aaactatcat ctggatccgtat ctgtcaccatctca tcttaaaaaa cttgtgaaaa tacgtaatcc tcaggagact tcaattaggt ataaatacca gcacagag gaggatcctgcagcacattgttct gatcatctga agatcag – 3`

Figure 2: DNA sequences studied. Yellow = methylation sites, green = forward primer, red = reverse primer.

Separation Medium for CE:

Once the methylation reactions were completed, the methylated DNA strands were analyzed via capillary electrophoresis. As stated before, the methylated DNA was separated from non-methylated DNA on the basis of increased hydrophobicity using different polymers of varying degrees of hydrophobicity within the capillaries. The polymer separation mediums used in this study were synthesized from monomers of varying degrees of hydrophobicity, and the structures of these monomers can be observed in Figure 3.

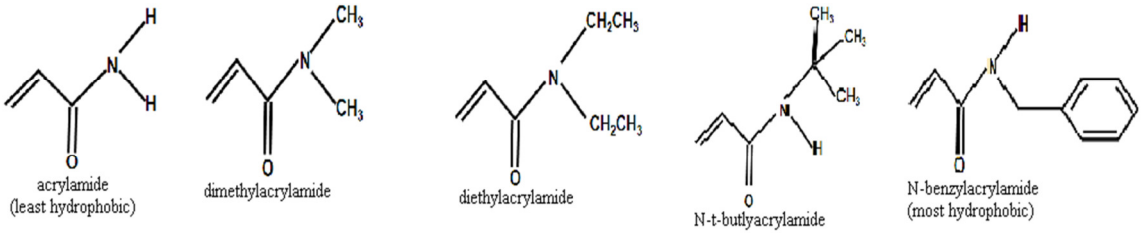

Figure 3: Structure of monomers that are polymerized to form the polymer separation matrix arranged in order of increasing hydrophobicity from left to right.

Polymers made of PA, PDMA, and PDEA were the polymers for which results were obtained in this study. These polymers are synthesized via free – radical addition polymerization in which the reaction is initiated by the action of free radicals, which are electrically neutral species with an unshared electron. More hydrophobic monomers, N-t-butylacrylamide and N-benzylacrylamide, are not able to be polymerized in this way; polymers consisting of these monomers are synthesized via micellar polymerization. In this copolymerization process, the hydrophobic monomer (N-t-butyl- or N-benzyl-) is solubilized within the hydrophobic interior of surfactant micelles, whereas the hydrophilic monomer (acrylamide) is dissolved in the hydrophilic aqueous continuous medium. This aqueous surfactant ensures the solubilization of the hydrophobic monomer within the micelle.[2] Different ratios of these monomers were used to create copolymers of varying hydrophobicities in order to determine the most effective

combination that can be used to detect DNA methylation. The copolymers were diluted to 3% by mass in 1X TBE buffer, which is also the running buffer used in CE. The conditions under which capillary electrophoresis was conducted are listed below in Table 1.

Table 1: Experimental conditions for capillary electrophoresis

CE Conditions		
Parameter	Value	Range
Oven Temperature	25	18-65 Deg. C
Polymer Filling Volume	4840	4840-38000 steps
Current Stability	5	0-2000 uAmps
PreRun voltage	15	0-15 kVolts
PreRun Time	180	1-1000 sec
Injection Voltage	15	1-15 kVolts
Injection Time	30	1-600 sec
Voltage Number of Stepss	40	1-100 nk
Voltage Step Interval	15	1-60 sec
Data Delay Time	1	1-3600 sec
Run Voltage	15	0-15 kVolts
Run Time	2100	300-14000 sec

Results and Discussion:

The potential for detection of DNA methylation based on hydrophobic interactions with a separation medium in capillary electrophoresis was investigated by using different combinations of varying degrees of hydrophobic copolymers. Results were obtained for polymer and copolymer separation mediums made up of 100% PDMA, 50% PDMA / 50% PDEA, and 30% PDMA / 70% PDEA. During each CE run, one capillary contained pure non-methylated DNA, one capillary contained pure methylated DNA, and the remaining two capillaries contained an evenly distributed mixture of methylated and non-methylated DNA. The electropherograms for each of the three combinations of copolymers are depicted in Figures 4-6.

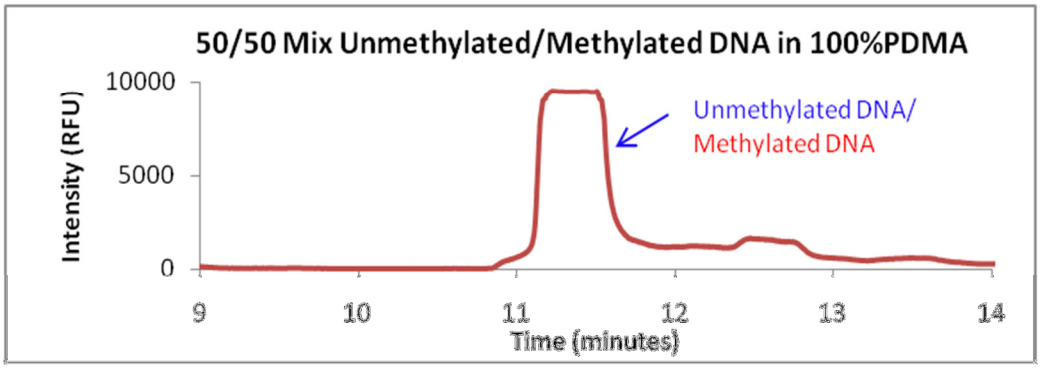

Figure 4: No separation was produced between unmethylated and methylated DNA in 100% PDMA.

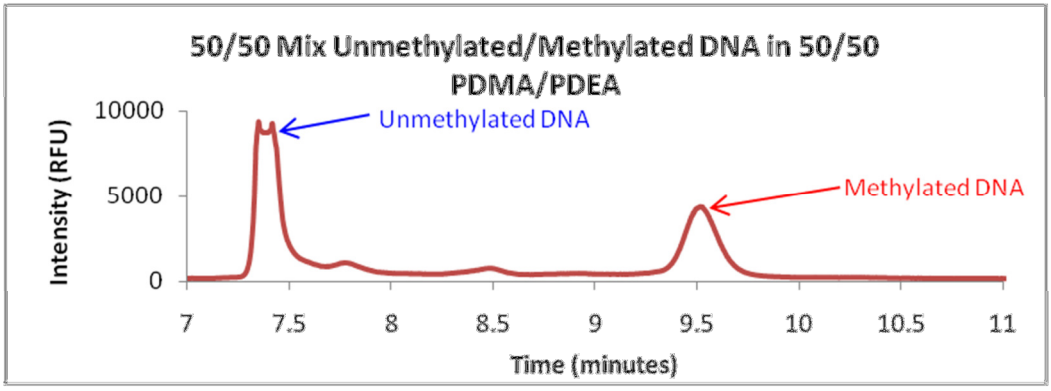

Figure 5: 50/50 PDMA/PDEA polymer produced separation between unmethylated and methylated DNA; however, repeated trials with this polymer produced inconsistent results.

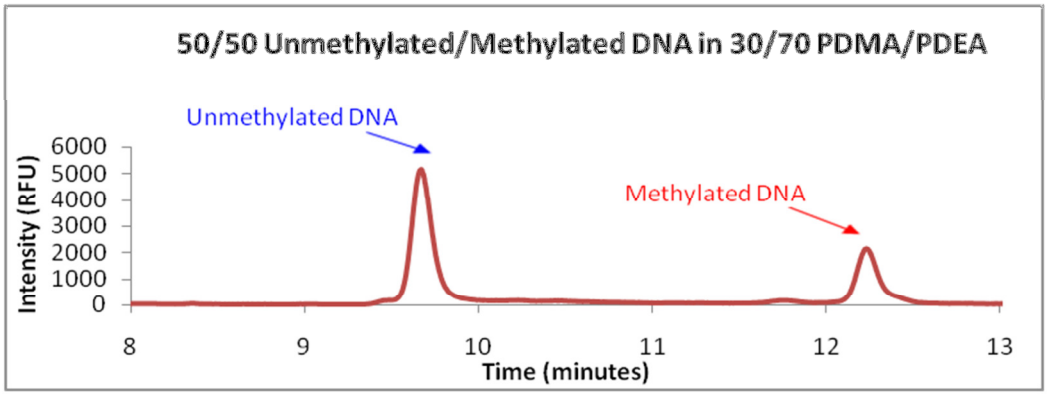

Figure 6: 30/70 PDMA/PDEA produced the effective separation of unmethylated and methylated DNA.

As can be seen in Figure 4, the 100% PDMA separation polymer produced no separation between methylated and non-methylated DNA. Figures 5 and 6 illustrate the separation between methylated and unmethylated DNA in 50/50 and 30/70 PDMA/PDEA polymer respectively. Although these two electropherograms demonstrate similar separation efficiency, the 30/70 PDMA/PDEA polymer produced far more consistent separation between the two types of DNA than the 50/50 PDMA/PDEA polymer when the experiments were repeated.

Analysis was done on each of the polymers that produced separation between methylated and unmethylated DNA. This analysis included comparisons of resolution, time spacing, peak width, and total analysis time. Resolution was determined using equation 2:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2} \quad (2)$$

where t = time at which the electrophoresis peak was observed and w = width of the peak at half the maximum. The 100% PDMA polymer demonstrated a resolution value of 0 because this polymer produced no peak separation. The resolution values for 50/50 and 30/70 PDMA/PDEA polymer are illustrated below in Figure 7.

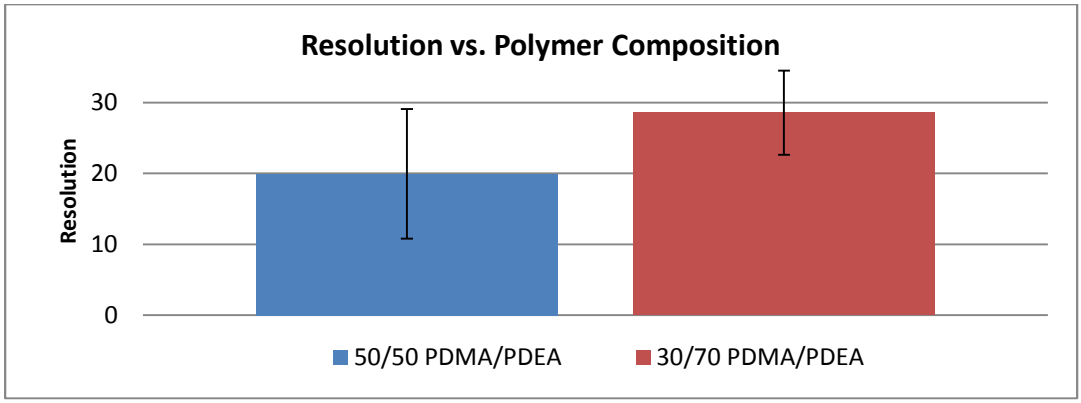

Figure 7: Comparison of resolution values of 50/50 PDMA/PDEA and 30/70 PDMA/PDEA polymer

The efficiency of separation in a CE process is measured by the resolution value. A more efficient peak separation results in higher resolution values. A higher resolution value for the 30/70 PDMA/PDEA polymer can be observed in this figure; however, the resolution values for each of the copolymers were not concluded to be statistically different due to the standard deviations of each set of results. When the CE experiments were performed, a preliminary run was conducted followed by a minimum of four repeat runs on each sample. The CE results for the separation of DNA were much more consistent throughout each one of these repeat experiments for electrophoresis conducted using 30/70 PDMA/PDEA polymer. This improved consistency of results is demonstrated in the comparison of the standard deviation of resolution values for the 50/50 and 30/70 polymer. The average resolution value for the 50/50 polymer was ~20 with a standard deviation of ~9.2 while the average resolution value for the 30/70 polymer was ~29 with a standard deviation of ~5.9. Although no definitive conclusions could be drawn from the resolution values that were obtained, the difference in the standard deviations of each set of results demonstrated the ability of the 30/70 polymer to provide more consistent peak separation.

Further analysis was done to evaluate each parameter of the resolution value equation (time spacing and peak width) and the average total analysis time in each separation medium. Time spacing between electrophoretic peaks is an important parameter in the determination of the effectiveness of separation in CE. As the time spacing between peaks in a CE process increases, the resolution value will increase, resulting in a more efficient peak separation. The time spacing between electrophoresis peaks for each of the polymer compositions that produced separation can be observed below in Figure 8. The average time spacing between the methylated and unmethylated DNA peaks for CE were a nearly identical 2.38 minutes for CE conducted

using 50/50 polymer composition and 2.33 minutes for the 30/70 polymer composition. The standard deviation for 50/50 polymer was ~0.89 compared to a standard deviation of ~0.24 for the 30/70 polymer. The smaller standard deviation of time spacing in the 30/70 polymer further demonstrates the improved consistency of results that can be obtained using this composition.

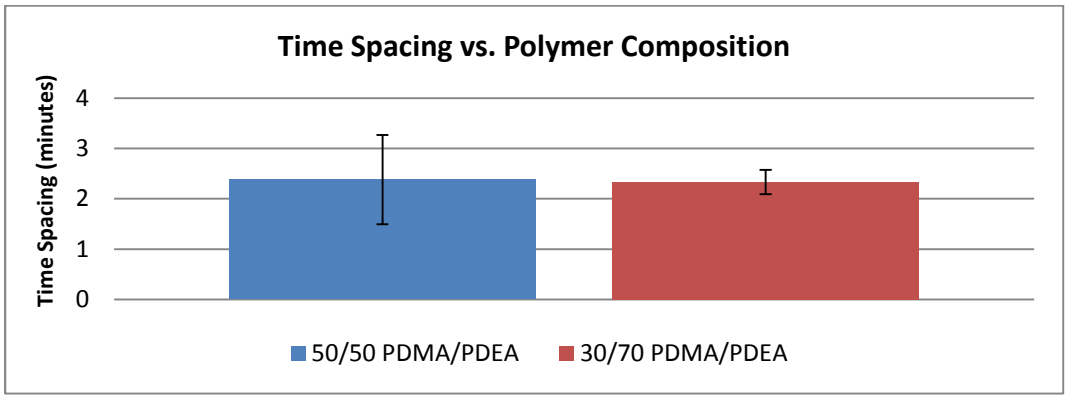

Figure 8: Time spacing between electrophoresis peaks for CE conducted in 50/50 and 30/70 PDMA/PDEA polymer.

The average peak width versus polymer composition is illustrated below in Figure 9. Peak width is a parameter that measures the time it takes for a sample to completely pass through the detection zone in CE. As total peak width decreases, the resolution value increases, which signifies a more efficient separation process. In this study, it was expected that stronger hydrophobic interactions between the 30/70 polymer and methylated DNA would produce a larger total peak width than the 50/50 polymer due to slower movement through the detection zone. This assumption was contradicted, however, when comparing the average total peak width in each of the polymers. The average peak width for 50/50 PDMA/PDEA polymer was ~0.27 minutes with a standard deviation of ~0.12 minutes, and the average peak width for 30/70 PDMA/PDEA polymer was ~0.17 minutes with a standard deviation of ~0.044 minutes. Once again, the values obtained for the total peak width in each of the polymers could not be concluded to be statistically different, but the 30/70 polymer produced more consistent results.

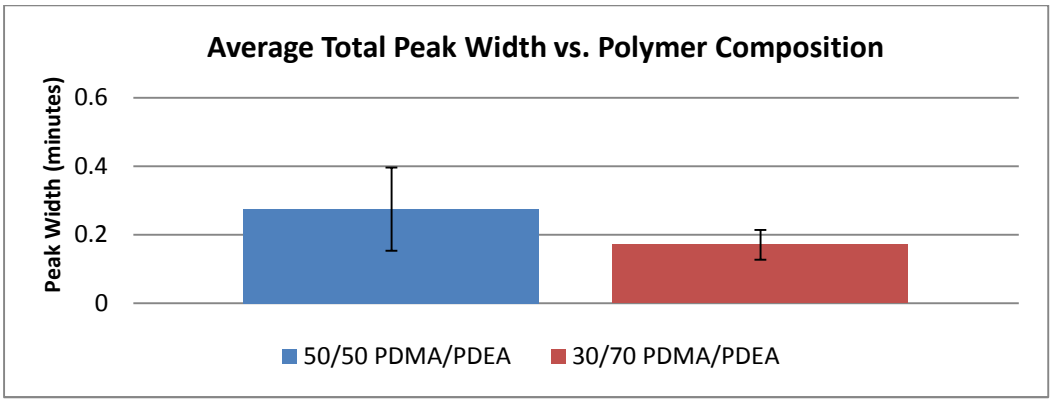

Figure 9: Effect of polymer composition on electrophoresis peak width

Further analysis was performed to compare the average peak width of the unmethylated and methylated DNA peaks individually in each of the different polymers. This analysis was done in order to determine the effect of the varying polymer hydrophobicity on the speed at which the unmethylated and methylated DNA moved through the detection zone. As can be observed in Figure 10, the results obtained from this analysis once again contradict with the assumption that the 30/70 polymer would produce a larger peak width for the methylated DNA. The unmethylated DNA peak widths were statistically the same, but the peak width for methylated DNA was statistically larger in 50/50 PDMA/PDEA. The average peak widths for unmethylated DNA were ~0.097 minutes in 50/50 polymer and ~0.087 minutes in 30/70 polymer with standard deviations of ~0.058 and 0.019 minutes respectively. The average peak widths for methylated DNA were ~0.18 minutes in 50/50 polymer and ~0.083 minutes in 30/70 polymer with standard deviations of ~0.071 and 0.024 minutes respectively.

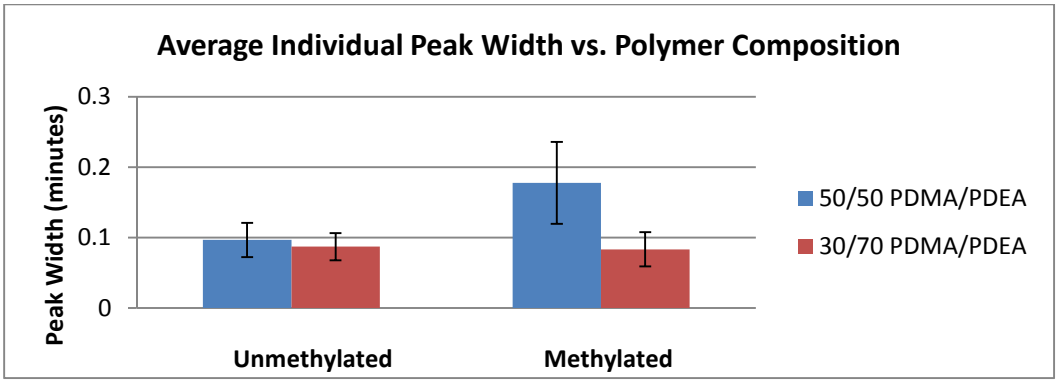

Figure 10: Comparison of average peak widths of unmethylated and methylated DNA peaks in each polymer.

It is difficult to determine the exact reason for these results because there are many factors that have an effect on the CE process. One probable cause for larger peak widths in the 50/50 polymer could have been the uneven distribution of monomers throughout the copolymer. This uneven distribution could have resulted in pockets of the more concentrated hydrophobic monomer, DEA, being formed throughout the copolymer matrix. If the 30/70 polymer possessed a more even distribution of monomers than the 50/50 polymer, methylated DNA migration time could have been slower through the detection zone in the 50/50 polymer as a result of stronger interactions with the highly concentrated pockets of PDEA. This, however, is only speculation, so more tests are needed to determine the cause of these results.

The total analysis time is the total time it takes for all of the analytes to move through the detection zone in CE. This value can be determined by observing the time on an electropherogram at which the last peak returns to an intensity value of zero. Analysis of this parameter is important in order to further justify the separation efficiency of a CE process. It is a primary goal to achieve reasonable analysis times (~5-15 minutes) while maintaining suitable resolution values. The average total analysis time for the 50/50 PDMA/PDEA polymer was ~11.04 minutes with a standard deviation of ~2.09 minutes, and the average total analysis time

for the 30/70 PDMA/PDEA polymer ~11.14 minutes with a standard deviation of ~1.57 minutes. Although these values were statistically equivalent, the short amount of time that was required for analysis demonstrated the potential for this detection method to be a very efficient process because of the high resolution values that were obtained.

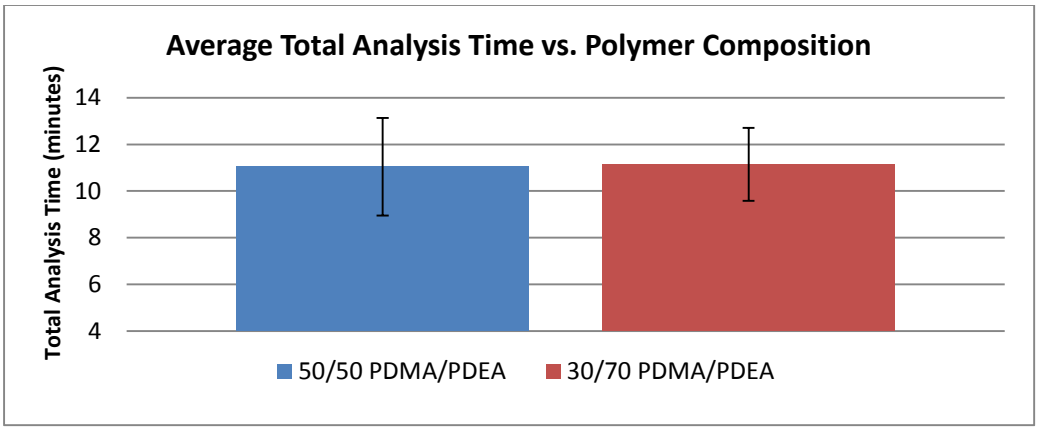

Figure 11: Effect of polymer composition on total CE analysis time.

The comparisons drawn between the CE results of the 50/50 and 30/70 PDMA/PDEA polymer demonstrate the higher separation efficiency that was achieved using the 30/70 polymer composition. 30/70 PDMA/PDEA was a more hydrophobic polymer than the 50/50 polymer composition. This increased hydrophobicity resulted in more efficient separation between unmethylated and methylated DNA due to the stronger hydrophobic interactions between the polymer and the methylated DNA. The values for resolution, time spacing, total peak width, and analysis time as a function of polymer composition did not differ statistically, so conclusions could not be obtained through the comparison of these values in each polymer. Nevertheless, a comparison of the standard deviations from the average values in each polymer composition demonstrated that the 30/70 composition provides more consistent and reliable results.

Overall, the results obtained in this study indicate that the separation efficiency of methylated DNA from unmethylated DNA increases as a more hydrophobic separation medium is used in CE. The use of 30/70 PDMA/PDEA polymer provided more consistent results than 50/50 PDMA/PDEA polymer when the experiments were repeated. This higher consistency and efficiency provided more conclusive detection of methylated DNA when it was mixed with unmethylated DNA.

Conclusions and Future Work:

The results obtained in this study suggest that the interactions of more hydrophobic methylated DNA and a hydrophobic separation medium in capillary electrophoresis is a viable target for detection of altered levels of methylation in DNA. As the hydrophobicity of the separation polymer was increased, the separation efficiency improved, which allowed more definitive detection of methylated DNA from unmethylated DNA. The methylated DNA used in this study only contained one methylation site for BAM H1 methyltransferase, so the degree of methylation of the DNA was very low. The high resolution values that were obtained using 30/70 PDMA/PDEA polymer indicate that a polymer with this degree of hydrophobicity has the potential to be used to effectively detect altered degrees of methylation in DNA.

It is recommended that further research be completed to determine detection efficiency using polymers of increased hydrophobicity, such as N-t-butylacrylamide and N-benzylacrylamide. Based on the results obtained in this study, it can be assumed that the increased hydrophobicity of these separation polymers will lead to better separation of methylated and unmethylated DNA in capillary electrophoresis, but it might also result in much longer analysis times that could make the use of these polymers less efficient. It is also

recommended that further research be completed to observe the effects of using different degrees of methylated DNA on the separation abilities of each polymer. In this study, DNA with only a slight degree of methylation was separated from unmethylated DNA in CE, but no results were obtained to determine the effects of using a mixture of varying degrees of methylated DNA on the separation abilities of each polymer.

Detection of altered patterns of DNA methylation in humans has attracted significant attention throughout the last few years due to the potential reversibility of DNA methylation, which suggests a viable target for the treatment of certain types of cancer.[9] This study indicates that detection of DNA methylation using hydrophobic interactions in capillary electrophoresis has the potential be an efficient and effective method. Ultimately, the goal of this research is be able to optimize this method to scan a sample of a person's genomic DNA and detect abnormal degrees of methylation. This could result in more effective treatments of types of cancer that are the result of altered DNA methylation patterns.

Acknowledgements:

I would like to gratefully acknowledge Dr. Christa Hestekin for allowing me this opportunity and for advising me throughout this project. I would also like to thank Elizabeth Pryor, Sowmya Krothapalli, and Alice Jernigan for all of the technical support they provided during this study. Financial support was provided by the University of Arkansas Honors College.

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